Organic Process Research & Development **2005**, *9,* 943–950

Communication to the Editor

An In-Line Study of Oiling Out and Crystallization

Emilie Deneau[†] and Gerry Steele*[,‡]

University of Pau, Ecole Nationale Supérieure en Génie des Technologies Industrielles, Pau, France, and AstraZeneca R&D Charnwood, Process Engineering, Process Research and Development, Bakewell Road, Loughborough, Leicestershire, LE 11 5RH UK

Abstract:

The oiling out and crystallization of a pharmaceutical compound from a water−ethanol mixture has been studied using a range of in-line process analytical techniques in order to more fully understand the phenomenon and build in control of its crystallization. Mechanistically, oiling out was found to follow a number of steps. It was shown that, in a well-mixed jacketed vessel, the small oil droplets formed on cooling a supersaturated solution grew and then coalesced into larger droplets, which subsequently crystallized. Cooling a static supersaturated solution of the compound resulted in two liquid layers, which if undisturbed were relatively stable. Chromatographic analysis of these layers showed that they contained each of the components, but in different amounts, and that crystallization occurred in the compound rich phase. In situ measurements showed that the temperature−solubility and the cloud point curve were well separated at lower temperatures but merged at higher temperatures. Identification of a metastable zone allowed a seeding strategy to be pursued, and by using a simple factorial experimental design, conditions that eliminated the oiling of the compound were determined.

Introduction

There are a number of ways in which a compound can be crystallized from a solution, e.g., reaction, cooling, precipitation (drown out), evaporation, or a combination of these techniques. However, it is sometimes observed that, instead of crystallizing from a supersaturated solution, droplets of a second liquid phase separate. This phenomenon is frequently termed oiling out[1] or liquid−liquid demixing.[2] From a thermodynamic point of view, phase separation normally only takes place when the Gibbs free energy of mixing (eq 1) is greater than zero, i.e., $\Delta G_{mix} > 0$. In eq 1 μ_A^* and μ_B^* are the reference chemical potentials of components A and B, respectively.

$$\Delta G_{mix} = x_A(\mu_A - \mu_A^*) + x_B(\mu_B - \mu_B^*) \quad (1)$$

However in some systems, such as those exhibiting oiling out, phase separation can occur when ΔG_{mix} is negative with respect to the unmixed components. As shown in Figure 1 for the two component system A and B exhibiting oiling out, the net ΔG_{mix} is negative for all of their mixtures; however, ΔG_{mix} can be minimized at two compositions, x_{AB} and x_{BA}, such that a miscibility gap is generated. At these compositions, the net overall ΔG_{mix} is lower when compared to any mixture between x_{AB} and x_{BA}, and these are known as the binodal points for mixtures of A and B.

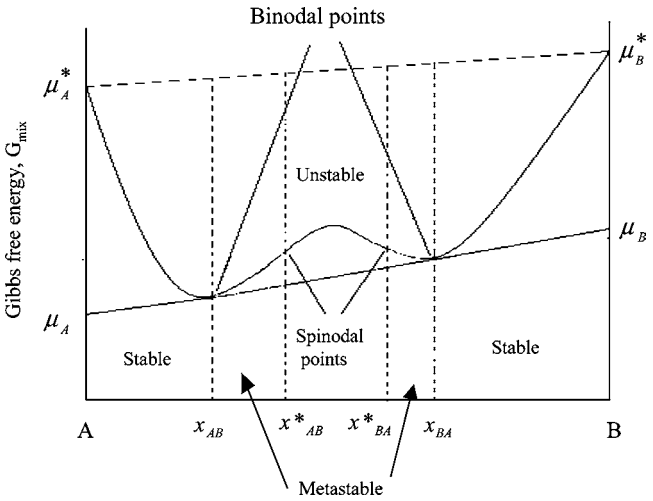

Figure 1. **Gibbs free energy function exhibiting liquid−liquid miscibility gap.**

The key thermodynamic property of the binodal points is that the chemical potential of each species is equal in both phases. Thus mixtures of A and B whose compositions are lower than x_{AB} and higher than x_{BA} are stable and do not phase separate. Between x_{AB} and x^*_{AB} and x_{BA} and x^*_{BA}, the system is metastable; i.e., phase separation can occur, but an energy barrier has to be overcome to initiate a new phase. The points x^*_{AB} and x^*_{BA} are known as the spinodal points, beyond which the solution is unstable and will spontaneously divide into the two liquid phases of equal chemical potential. This process is termed spinodal decomposition where separation proceeds without any thermodynamic barrier. It should be noted that partial phase immiscibility is a function of

* To whom correspondence should be addressed. E-mail: gerald.steele@astrazeneca.com. Telephone: +44 (0) 1509 644695. Fax: +44 (0) 01509 644.
[†] University of Pau.
[‡] AstraZeneca R&D Charnwood.

(1) Bonnett, P. E.; Carpenter, K. J.; Dawson, S.; Davey, R. J. *Chem. Commun.* **2003**, 698.
(2) Lafferrére, L.; Hoff, C.; Veesler, S. *J. Cryst. Growth* **2004**, *269*, 550.

10.1021/op050107c CCC: $30.25
Published on Web 10/28/2005

temperature. For systems in which the degree of immiscibility decreases with increasing temperature, an upper consolute temperature (UCT) is defined as that temperature at which complete miscibility occurs.

In practice oiling out is undesirable since, e.g., the oil phase is often a good solvent for impurities and if it subsequently crystallizes it can render the whole crystallization process worthless in delivering purification. Furthermore, it has been noted that scale-up of such a system will be problematic, since the power criterion of constant power per unit volume will produce smaller droplets due the higher tip speeds of impellers used in larger vessels.[3] Until recently, there were only a few references in the literature with regard to organic small molecules to help process chemists and engineers to understand and ultimately control this phenomenon.[1−4]

In this paper, a number of process analytical technologies (PAT) have been used to follow the oiling out and crystallization of a pharmaceutical compound. PAT techniques are becoming increasingly important in the pharmaceutical industry to aid process understanding and problem solving. Indeed, the Food and Drug Administration (FDA) see PAT as systems for design, analysis, and control of manufacturing processes,[5] and papers describing the use of PAT for crystallization development have recently been published.[6−7]

The PAT techniques used in this study were focused beam reflectance measurements (FBRM, commonly known as Lasentec), particle vision monitor (PVM), and attenuated total reflectance ultraviolet/visible (ATR UV/vis) spectroscopy. In FBRM studies, a laser beam is projected through a sapphire window of the FBRM probe and highly focused just outside the window surface (0.2−0.8 μm). This is then moved so that it follows a path around the circumference of the sapphire window. The focused beam moves at a high velocity (4500rpm or 2 ms^{-1}) so that particle motion is insignificant to the measurement. As the particles pass by the window surface, the focused beam intersects the edge of the particle, which then backscatters the laser light. This continues until it reaches the opposite edge. The backscattered light is detected by the FBRM probe and converted into an electronic signal, and the instrument measures the time period of the backscatter from edge to edge, which, when multiplied by the scan speed, results in a distance known as the chord length. This is defined as a straight line between any two points on the edge of a particle. In a typical experiment, the probe measures tens of thousands of chords per second. The operation of the FBRM and its use in the pharmaceutical and fine chemical arenas have been discussed by a number of authors, and it is considered to be the industry standard for the in situ measurement of particle size.[7−15]

PVM is a high-resolution video microscope, which shows an image of crystals that pass the window every second. It uses six independent laser beams to light up an area within the slurry. Backscattered light is seen by a sensor and made into an image with an area of 860 × 645 μm^2. The PVM can detect crystals as small as 5 μm.[11]

The use of ATR UV/vis technique for measuring supersaturation and desupersaturation kinetics has been reported.[16−17] As with other ATR methods, this technique relies on the occurrence of a short-lived (evanescent) wave in the suspension medium where the UV/vis radiation is propagated due to internal reflection. Since the penetration of this wave is of the order of the wavelength of the radiation, it is assumed that there is no interaction with the crystals and hence can be used to measure the solution concentration of a solute. Clearly the use of an in-line probe has the advantage over an at-line technique such as HPLC for monitoring the supersaturation; i.e., it avoids sampling at elevated temperature and can monitor the solute concentration during crystallization.

Materials and Methods

The pharmaceutical studied in this investigation, compound A, cannot be identified for commercial reasons. It was produced by Process Research & Development, AstraZeneca R&D Charnwood, Loughborough and had a purity of 99.85%. Ethanol, analytical reagent grade, was obtained from Fisher Scientific UK, Loughborough UK, and the water used was generated from a MilliQ reverse osmosis unit.

Oiling out and crystallization experiments were mainly performed in a 250 mL jacketed reactor (Radleys Ltd, Saffron Walden, Essex, UK) fitted with a retreat curve impeller in glass with three blades. The speed of the agitator was regulated with an IKA EUROSTAR power control-visc. device (IKA Werke GmbH & Co. Staufen, Germany) and a condenser was fitted to the reactor to prevent the loss of the solvent on heating. The temperature was maintained using a Huber Ministat unit (Radleys Ltd, Saffron Walden, Essex, UK), which was controlled with a PC using the software package Labworldsoft (IKA Werke GmbH & Co. Staufen, Germany). A temperature probe fitted in the reactor recorded the changes in temperature imposed by the Huber (T_r). The reactor values could be compared to the set temperature (T_{set}) and the jacket temperature (T_j).

A PI 14/206 Lasentec probe (Mettler-Toledo Autochem Ltd, Leicester, UK) was used to follow the oiling out and crystallization of compound A and to determine its cloud and clear point curves in 50:50 v/v ethanol/water. The data

(3) Veesler, S.; Lafferrére, L.; Garcia, E.; Hoff, C. *Org. Process Res. Dev.* **2003**, *7*, 983.
(4) Lafferrére, L.; Hoff, C.; Veesler, S. *Cryst. Growth Des.* **2004**, *4*, 1175.
(5) Yu, L. X.; Lionberger, R. A.; Raw, A. S.; D'Costa, R. D.; Wu, H.; Hussain, A. J. *Adv. Drug Delivery Rev.* **2004**, *56*, 349.
(6) Birch, M.; Fussell, S. J.; Higginson, P. D.; McDowall, N.; Marziano, I. *Org. Process Res. Dev.* **2005**, *9*, 360.
(7) Barrett, P.; Smith, B.; Worlitschek, J.; Bracken, V.; O'Sullivan, B.; O'Grady, D. *Org. Process Res. Dev.* **2005**, *9*, 348.
(8) Dowding, P. J.; Goodwin, J. W.; Vincent, B. *Colloids Surf., A* **2001**, *192*, 5.
(9) Heath, A. R.; Fawell, P. D.; Bahri, P. A.; Swift, J. D. *Part. Part. Syst. Charact.* **2002**, *19*, 84.
(10) Ruf, A.; Worlitschek, J.; Mazotti, M. *Part. Part. Syst. Charact.* **2000**, *17*, 167.
(11) Kougoulos, E.; Jones, A. G.; Jennings, K. H.; Wood-Kaczmar, M. W. *J. Cryst. Growth* **2005**, *273*, 529.
(12) Barrett, P.; Glennon, B. *Chem. Eng. Res. Des.* **2002**, *80*, 799.
(13) Togkalidou, T.; Tung, H.-S.; Sun, Y.; Andrews, A. T.; Braatz, R. D. *Ind. Eng. Chem. Res.* **2004**, *43*, 6168.
(14) Doki, N.; Seki, H.; Takano, K.; Asatani, H.; Yokota, M.; Kubota, N.; *Cryst. Growth Des.* **2004**, *4*, 949.
(15) O'Sullivan, B.; Barrett, P.; Hsiao, G.; Carr, A.; Glennon, B. *Org. Process Res. Dev.* **2003**, *7*, 977.

were collected every 15 s in the 1−1000 μm, 90 log channels mode. To visualize the processes occurring in situ, a particle vision monitor (PVM, Mettler-Toledo Autochem Ltd, Leicester, UK) was used in a 700 mL jacketed vessel with a specially designed lid to accommodate the PVM and other probes (Radleys Ltd, Saffron Walden, Essex, UK). Heating control and the temperature programme were the same as those used for the 250 mL reactor. The speed of the agitator was regulated with a Heidolph RZR2021 motor (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany).

A Zeiss MCS 500 spectrometer with a Hellma 661.812 Attenuated Total Reflection (ATR) UV/vis probe (both supplied by Clairet Scientific, Northhampton, UK) was used to collect information about the change in absorbance of the compound A as it was heated and cooled in the 50:50 v/v ethanol/water mixture. For operational reasons, air was chosen as the reference measurement. The spectral range was 199−404 nm, and a spectrum of the solution was recorded every 60 s. Measurements were collected at the λ_{max}, 250 nm, and were uncorrected for temperature changes.

To determine the concentration of compound A in the two phases, high performance liquid chromatography (HPLC) and gas chromatography (GC) were performed on samples quickly removed from the two layers. GC analyses were performed using a Hewlett-Packard 6890 GC (Aglilent Technologies, UK Stockport, UK) in constant flow mode. The column was a DB-624 20 m long × 18 mm × 1.00 μm film thickness. The carrier gas was helium maintained at 100 °C, and its flow was 1.1 mL/min. At the inlet the temperature was 250 °C, the split injection was 1 μL, and the split ratio was 10:1. At the detector the temperature was 300 °C, the airflow was 300 mL/min, the hydrogen flow was 30 mL/min, and the make up gas was 30 mL/min. At the beginning of a run, the temperature in the oven was held at 40 °C for 2 min, then increased to 100 °C at a rate of 30 °C/min and finally brought to 250 °C at 50 °C/min. The sample frequency was 25 Hz, and a run lasted 7 min. HPLC was performed using an Agilent 1100 Series HPLC (Aglilent Technologies Stockport, UK). The column used was a Genesis C18 3 μm, and its thermostat temperature was 40 °C. The wavelength of maximum absorbance (λ_{max}) of compound A was 250 nm. This value was chosen as the wavelength of detection, and the pressure range was 0 to 400 bar. A gradient method was chosen using two mobile phases at a flow rate of 1 mL/min. During the analysis, mobile phase A consisted of water and 0.1% v/v trifluoroacetic acid (TFA), and the mobile phase B was a 90:10 acetonitrile/water solvent mixture with 0.1% v/v TFA. The injection volume was 5 μL, and after each injection the needle was washed with acetonitrile. The run time was 12 min.

An Olympus BX51 microscope (Olympus UK Ltd, Southall, UK) with a 3-CCD colour video camera (JVC model KY-F55B) was used for microscopic examination of the oil droplets and crystals. Images were analyzed with ImagePro Plus image analysis software (Media Cybernetics Inc, Silver Springs, USA).

Methodology Used To Investigate the Mechanism of Oiling Out. To determine the mechanism of oiling out, compound A and the solvent mixture were charged to the 250 mL reactor to provide 160 mL of a solution of 200 mg/mL compound A in 50:50 v/v ethanol/water. The temperature was held at 20 °C for 10 min, heated to 55 °C at 0.5 °C/min, and then held at this temperature for 15 min. The solution was then cooled to 5 °C at 0.2 °C/min and held at 5 °C for 10 h. The stirring rate was 450 rpm.

Table 1. **Factors and levels examined in the seeding study**

experiment number	size of seed (μm)	amount of seed (% w/w)	cooling rate (°C/min.)
1	20−45	1	0.1
2	20−45	1	0.2
3	20−45	1	0.5
4	20−45	5	0.1
5	20−45	5	0.2
6	20−45	5	0.5
7	250−500	1	0.1
8	250−500	1	0.2
9	250−500	1	0.5
10	250−500	5	0.1
11	250−500	5	0.2
12	250−500	5	0.5

Methodology Used To Determine the Temperature−Solubility and Liquid−Liquid Separation Curves of Compound A in 50:50 v/v Ethanol/Water. To determine the temperature−solubility curve of compound A and liquid−liquid separation boundary for this system, i.e., the clear and cloud points on heating and cooling, the following concentrations were studied: 225, 200, 175, 150, 125, 100, 75, and 50 mg/mL. A fresh solution (100 mL) was prepared for each concentration, and each experiment was conducted in quadruplicate to confirm the reproducibility of the results. For the first three concentrations, the solutions were heated to 55 °C, and the others, to 40 °C. The temperature was held at 20 °C for 10 min and then increased to the chosen value at 0.5 °C/min. The temperature was then held at this value for 20 min and cooled to 5 °C at 0.5 °C/min. Finally, the solution was left at 5 °C for 25 min.

Seeding Experiments. Once the temperature−solubility characteristics of compound A in 50:50 v/v ethanol/water had been established, seeding experiments were carried out using the FBRM and ATR UV/vis probes to monitor the changes in the solution during the experiments. From the temperature−solubility and liquid−liquid separation boundary curves it was decided to conduct this study with a solution of 125 mg/ml of compound A in 50:50 v/v ethanol/water. The seeds were introduced at 30 °C, which was within the area delimited by the solubility curve and the liquid−liquid-phase separation boundary. To assess the precise influence of each of the experimental parameters, e.g., size of the seed, amount of seed, and cooling rate, a simple three-factor, three-level design of experiments protocol was constructed. Table 1 represents the range and combination of parameters used.

A Gilsonic ultrasonic AutoSiever GA6 (Christison Scientific Equipment Ltd, Gateshead, UK) was used to produce seeds for the seeding experiments. Two sieve fractions were selected as seeds to investigate the effect of seed size on controlling the crystallization, i.e., 20−45 μm and 200−500 μm. The surface area of each size of seeds was determined

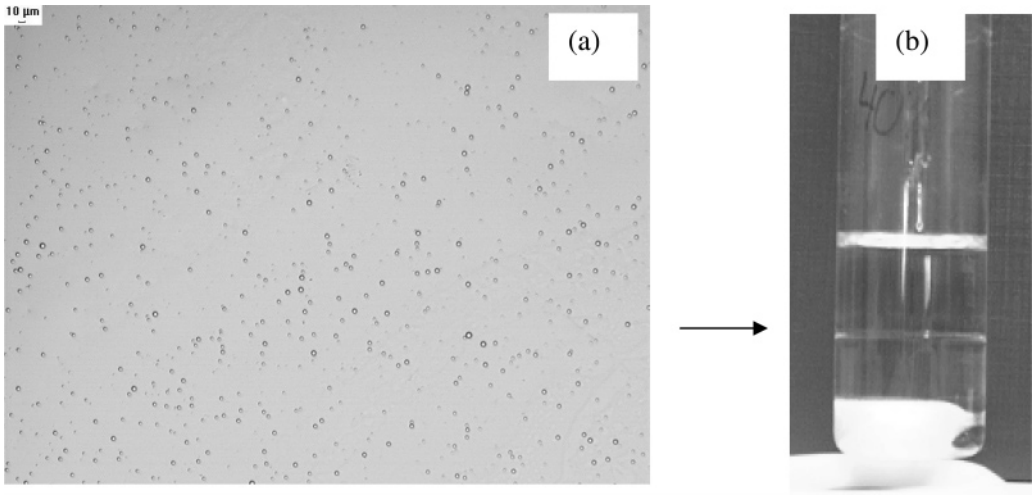

Figure 2. **Photograph of oil droplets of compound A initially produced on cooling (a) and after standing at room temperature (b).**

Table 2. **HPLC and GC analysis of the two layers shown in Figure 2b**

layer	% w/w compound A (HPLC)	% w/w ethanol (GC)	% w/w H_2O (by mass balance)
top	11.74	55.43	32.83
bottom	59.49	33.12	7.39

by BET measurements, using a Micromeretics Gemini III 2375 surface area analyser (Micromeretics UK Ltd, Dunstable, UK).

For each seeding experiment, 180 mL of a solution of compound A in 50:50 v/v ethanol/water was prepared. The solution was heated to 40 °C under agitated conditions (450 rpm), held at this temperature for 10 min, and then cooled to 5 °C at the chosen cooling rate. Finally, the solution was left at 5 °C. After the introduction of the seeds, a sample of the solution was taken and observed with a polarizing microscope.

Experimental Results and Discussion

Mechanism of Oiling Out. When an agitated solution of compound A in 50:50 v/v ethanol/water was cooled it initially separated as small droplets, which later coalesced and eventually the solution crystallized when agitated in the reactor. Figure 2a shows a photomicrograph taken after a hot solution of compound A was initially cooled (these droplets were not detectable using the PVM as they are below the limit of detection of this instrument). If the solution was not agitated on the other hand, the solution did not crystallize but separated into two distinct layers (Figure 2b).

Table 2 shows the HPLC analysis (for compound A) and GC data (for ethanol) of the two layers shown in Figure 2b.

From these data it can be seen that the bottom layer contained a higher proportion of compound A compared to the top layer. It should be noted that the proportions of the ethanol and water were also different in these layers. Although the relative concentrations in each phase were different (even though the same components were present), thermodynamically they have equal chemical potential. From a crystallization perspective, the probability of crystallization taking place in each phase is proportional to the kinetic rate for nucleation in each phase; i.e., although the two phases are in equilibrium, this does not imply that the crystallization kinetics are the same in each phase. However, for compound A it was observed that after the phases were disturbed by sampling for HPLC and GC analysis crystallization took place in the lower, compound A rich phase.

The combination of FBRM and ATR UV/vis and PVM measurements (Figures 3 and 4) showed that when the solution was cooled it spontaneously separated into two liquid phases. From these measurements, five main areas were identified on the plot: (a) Dissolution, (b) Oiling out, (c) Coalescence of oil droplets, (d) Release of compound A from the oil droplets and nucleation, (e) Crystal growth.

As expected, as the compound dissolved there was a decrease in the total number of counts measured by the FBRM and an increase in absorbance. There was a small event on heating, i.e., the FBRM showed a small increase and then decrease in total counts and the absorbance measurements also showed a peak. This was ascribed to be due to transient oiling on heating and appeared to be reproducible. On cooling, there was a sharp increase in the total number of counts recorded by the FBRM and a decrease in the UV absorbance measurements (Figure 3). This was due to formation of the oil droplets, and since the ATR UV/vis measures only measure a few microns into solution, the absorbance measurements were being made only in the compound A poor phase and not the droplets. As shown in Figure 3, the FBRM counts reached a maximum and then decreased whilst the absorbance data continued to fall. Examination of the PVM images (Figure 4a−c) showed that this corresponded to growth of the oil droplets via coalescence. It should be noted that the UV/vis data are uncorrected for any changes in the λ_{max} (small) or extinction coefficient that occur during the cool phase; however, the large increase then decrease in absorbance after 25 000 s for constant temperature indicates that the absorbance is a much stronger function of solution concentration than temperature.

The last portion of the measurements showed that there was an increase followed by a sharp decrease in both total

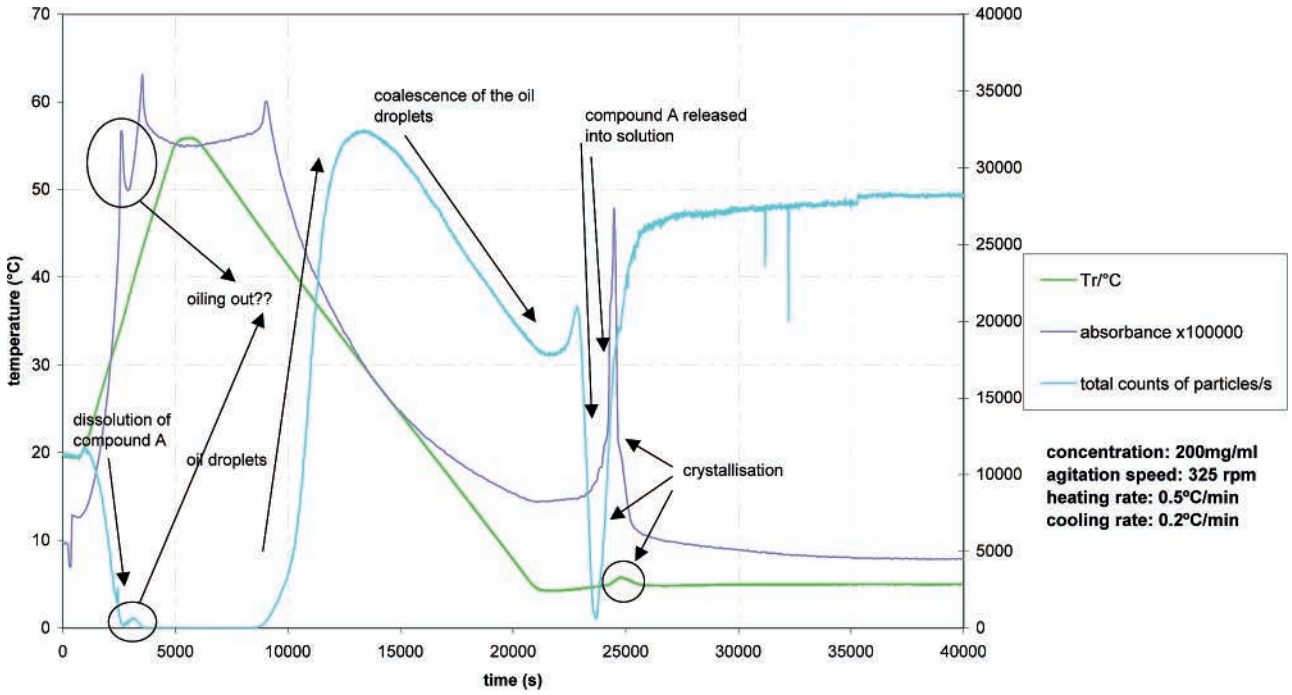

Figure 3. **Time course FBRM and ATR UV/vis data for the oiling and crystallization of compound A.**

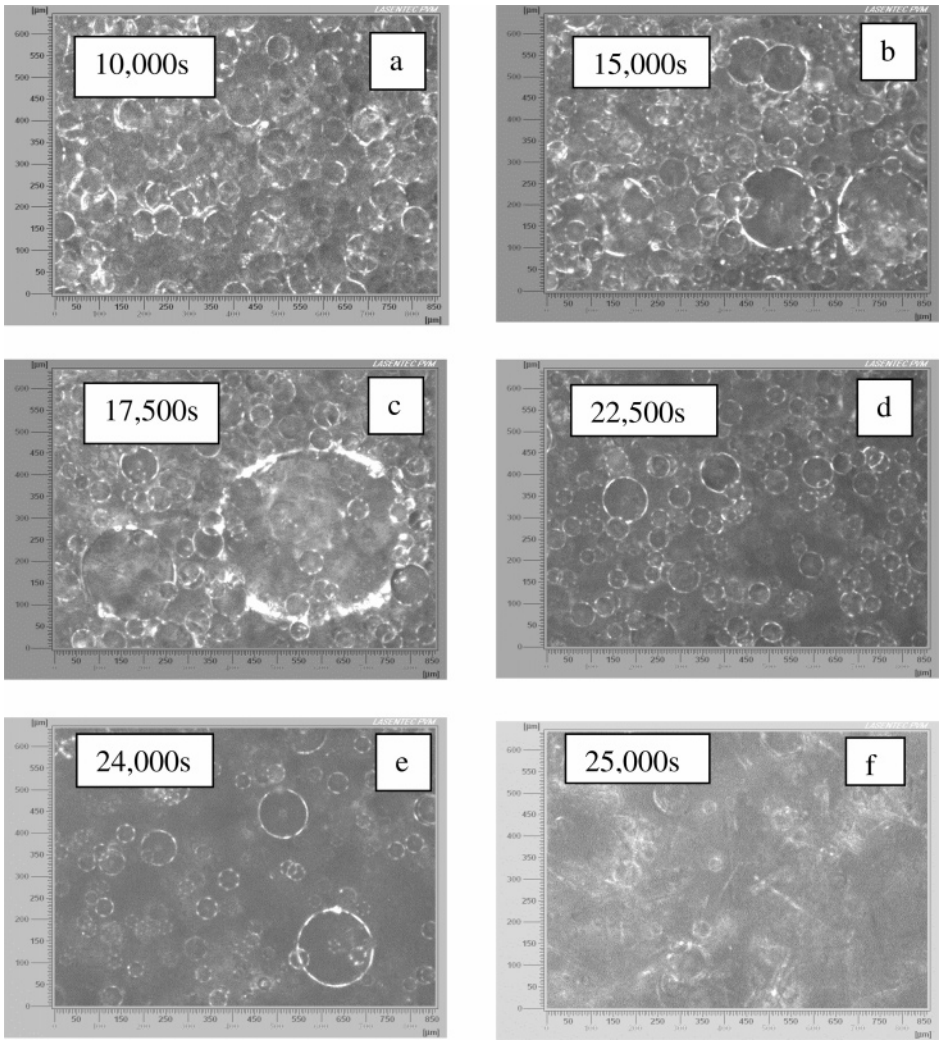

Figure 4. **PVM images of oiling and crystallization of compound A.**

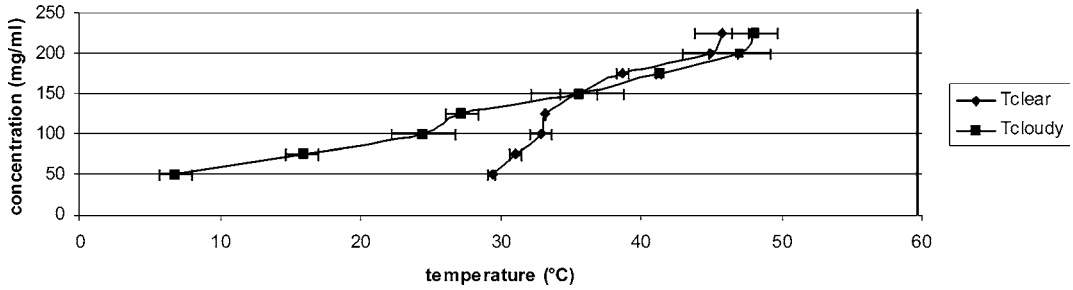

Figure 5. **Cloud and clear point measurements for compound A as a function of solution concentration.**

counts and absorbance measurements. This was interpreted as being due to a nucleation event and corresponded to a decrease in the size and number of oil droplets observed by the PVM (Figure 4d, e). The final part of the plot showed an increase in FBRM counts and a decrease in absorbance, implying that compound A left the droplets and transferred to the growing crystals. Figure 4f shows the presence of many acicular crystals with oil droplets also present. Crystallization was also detected by a small exothermic event in the reactor temperature curve.

Seeding Experiments. Using the method proposed by Barrett and Glennon,[12] FBRM was used to determine the temperature−solubility and oiling out curves in 50:50 v/v ethanol/water by noting the clear and cloud point temperatures for different concentrations of compound A (Figure 5). These data showed that at 50 mg/mL of compound A, the difference between the solubility and oiling out curves was relatively wide (∼25 °C). However, the MSZW was observed to decrease as the concentration of compound A increased until at 150 mg/mL and above the curves merged into a single curve within experimental error.

Since there was an identifiable metastable zone (MSZ), analogous to that seen in conventional cooling crystallizations, it implied that seeding could be used to induce crystallization before the solution oiled out. Seeding is a function of, e.g., the amount, size and activity of the seed, the saturation conditions at which the seed is added, and the cooling program used. To examine some of these variables, a simple factorial design (Table 2) was designed and implemented to examine the effect of seed size (20−45 and 250−450 μm), amount of seed, and the cooling rate. More sophisticated approaches to the seeding of pharmaceutical crystallizations have been reported.[18−21] BET measurements showed that the surface area of the small and large seeds were 0.41 and 0.12 m^2/g, respectively. The relatively small difference in surface area suggests that the seed band sizes should be treated as nominal, since the sieving of needlelike crystals may lead to errors if treated as an absolute measure of size, i.e., the needle can pass through the sieve aperture end on.

Figure 6a−d shows the FBRM data obtained for the factorial design experiments used to investigate the seeding of a solution of compound A. In Figure 6a, there was a rise in particle counts as the seeds were added which, when cooling was initiated, resulted in a small peak in the total number of counts observed. It is possible that it was due to aggregation of the seeds, but using PVM it was found to be due to some oiling. The total number of counts was seen to increase, reaching a plateau when crystal growth became the dominant process. The small peak in the FBRM data seen in Figure 6a was not observed when the 20−45 μm seed loading was increased to 5% w/w (Figure 6b). These data show that on cooling the seeded solution there was no oiling out, but simply crystal growth; i.e., oiling out was eliminated at all cooling rates investigated. The reduction in seed surface area available for crystal growth using 1% w/w seed of the 200−500 μm crystals coupled with a fast cooling rate (0.5 °C/min) produced a larger amount oiling out (Figure 6c). Increasing the seed loading to 5% w/w for the larger seeds had a positive effect on the extent of oiling out with the oiling out being suppressed at all cooling rates. Indeed, at the slowest cooling rate, it appeared to be completely eliminated.

It could be argued that the seeding temperature was too close to the oiling curve and that better results would have been obtained if seeding was performed at a lower supersaturation. For example, it has been stated that the best place for seeding is near to the solubility line and no more than a $^1/_4$ to a $^1/_2$ into the MSZ.[22] It is known that seeding has the effect of reducing the MZW compared to an unseeded crystallization;[23] therefore, seeding closer to the solubility line may reduce the amount of seed needed to control the oiling out of compound A. Furthermore, we used linear cooling ramps, and often nonlinear cooling ramps are used to maintain the supersaturation constant during crystallization runs.[19] Again, this may help to further control the oiling out of this compound.

For a cooling crystallization it has been argued that the amount of seed added is 0.1 to 0.3% w/w and that the size of seed should be between 0.1 and 0.3 of the final size of the crystals.[24] Indeed, the amount of seed to be added can be calculated from the initial and final solution concentrations.[14] In this case, despite the large differences in conditions under which the crystallizations were performed, there were no gross differences between the final crystal size distribu-

(16) Anderson, J. E.; Moore, S.; Tarczynski, F.; Walker, D. *Spectrochim. Acta* **2001**, *57*, 1793.
(17) Thompson, D. R.; Kougoulos, E.; Jones, A. G.; Wood-Kaczmar, M. W. *J. Cryst. Growth* **2005**, *276*, 230.
(18) Patience, D. B.; Dell'Orco, P. C.; Rawlings, J. B. *Org. Process Res. Dev.* **2004**, *8*, 609.
(19) Choong, K. L.; Smith, R. *Chem. Eng. Sci.* **2004**, *59*, 313.
(20) Chung, S. H.; Ma, D. L.; Braatz, R. D. *Can. J. Chem. Eng.* **1999**, *77*, 590.
(21) Lung-Somarriba, B. L. M.; Moscosa-Santillan, M.; Porte, C.; Delacroix, A. *J. Cryst. Growth* **2004**, *270*, 624.
(22) Beckmann, W. *Org. Process Res. Dev.* **2000**, *4*, 372.
(23) Mersmann, A. *Chem. Eng. Res. Des.* **1996**, *74*, 812.

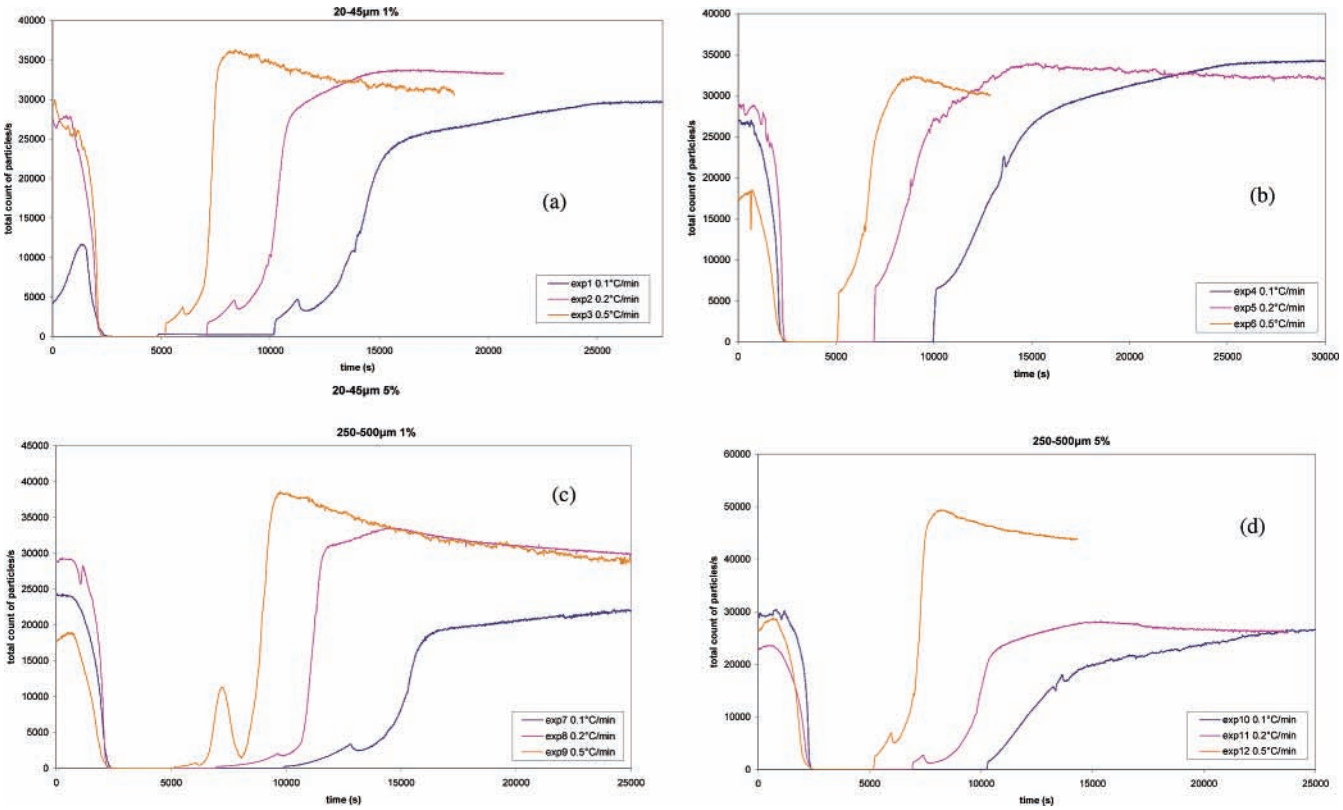

Figure 6. **FBRM total counts versus time data for the factorial investigation of the effect of seeding solutions of compound A.**

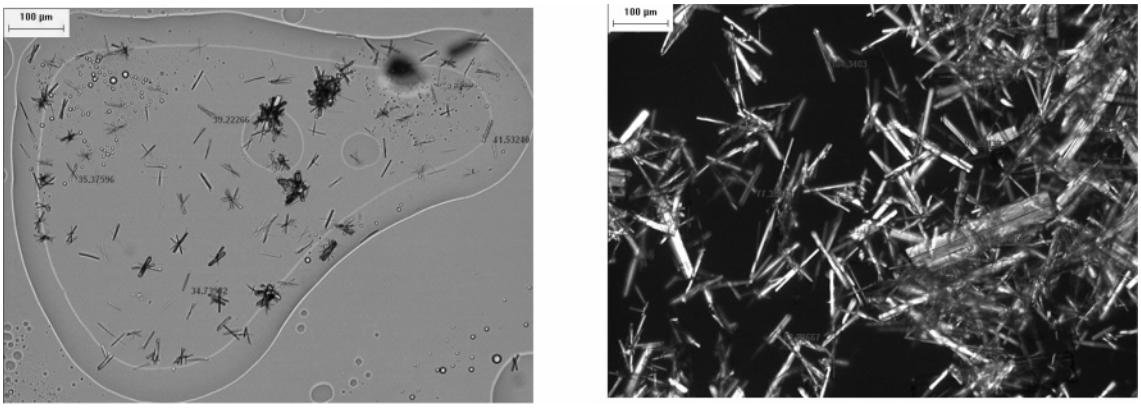

Figure 7. **Photomicrograph of seed crystals (20−45 μm size fraction) and crystals obtained from a crystallization shown in Figure 5b.**

tions (100−200 μm) of all crystallization runs (Figure 6). Figure 7 shows photomicrographs of (a) 20−45 μm seeds and (b) crystals obtained from these seeds at the 5% w/w loading (Figure 6b).

Conclusions

Supersaturated solutions of the pharmaceutical compound A in 50:50 v/v ethanol/water spontaneously separate into two liquid phases when they are cooled. Each phase contains ethanol, water, and compound A in different amounts with the oil droplets containing a higher proportion of compound A. The oil droplets grow through coalescence, which if allowed to stand without agitation form two liquid layers. If the system is agitated, however, the coalesced oil droplets sharply decrease in size and number as nucleation and crystal growth proceed.

The solubility curve and the liquid−liquid phase separation boundary of compound A in 50:50 v/v ethanol/water mixture is easily determined with the Lasentec FBRM probe. It revealed that at low concentrations the difference between the two curves is relatively wide; however, the gap between the two curves decreases as the concentration increases until the curves merge (within experimental error) at 150 mg/mL of compound A. This area between the solubility and oiling out curves is a metastable zone analogous to an ordinary cooling crystallization, which made it possible to control the oiling out of compound A by seeding. Oiling out is substantially suppressed by using a high seed loading (5% w/w) with a small crystal size (20−45 μm) and can be eliminated at all cooling rates investigated. Using a high load

(24) Heffles, S. K.; Kind, M. 1999, Proc.14th Int. Symp. Ind. Cryst., I. Chem. E UK, p 2234.

(5% w/w) of large seeds (250−500 μm) introduced at a slow cooling rate (0.1 °C/min) also eliminates the liquid−liquid phase separation. In all cases, seeding gives a good yield (between 81.4 and 93.2%), and the crystals are acicular and about 100−150 μm in length. This work shows that oiling out can be controlled and that by knowledge of the temperature−solubility and liquid−liquid separation curves the system can be brought back to a crystallization process that could be scaled. PAT techniques provided a valuable insight into the mechanism of oiling out of compound A and can be used to determine conditions for the further optimization of its crystallization.

Acknowledgment

We thank Dr. P. Bonnett (Syngenta) for very useful discussions on the phenomenon of oiling out, Parminder Ashan, Andrew Baker, and Nicholas Pedge, Analytical Chemistry, PR&D, for assistance with the chromatography and Uv/vis spectrometry. Mr Richard Bell, PAR&D, AstraZeneca R&D Charnwood, is thanked for determining the surface areas of the seed crystals. The authors would also like to thank the referees for their very helpful comments.

Received for review June 27, 2005.

OP050107C